REVIEW ARTICLE

Haemophilia: An Update

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Summary:

Haemophilia has been recognized a clinical entity since Biblical times when there was repeated history of death from circumcisional bleeding in male siblings. Recent advances in protein chemistry and recombinant DNA technology have produced a comprehensive account both of normal coagulation and of the molecular genetics of some type of haemophilia. Haemophilia is a hereditary coagulation disorders usually of male associated with serious bleeding which is transmitted by healthy women. It is caused by a reduction in the amount or activity of factor VIII. This protein serves as a cofactor for factor IX in the activation factor X in the coagulation cascade. Haemophilia A & Haemophilia B exhibit a wide range of clinical severity that correlate well the level of factor VIII activity. Those with less than 1% of normal activity develop severe disease; levels between 2% and 5% of normal are associated with moderate disease; and patient with 6% to 50% of activity developed mild disease. The variable degrees of factor VIII deficiency are largely explained by heterogenecity in the causative mutation. Several genetic lesions - deletions, nonsense mutations that create loop codons, splicing errors have been documented. Most severe deficiencies result from an unusual inversions involving X chromosomes that completely abolishes the synthesis of factor VIII. Haemophilia is inherited as X-linked recessive trait, and thus occurs in male and both homozygous and heterozygous female. Approximately 30% of patients have no family history; their disorder is presumably caused by new mutation.

In this review article we want to highlight the recent aspects of haemophilia including the occurrence of haemophilia in female, genetic causes of coagulation factor deficiency, carrier detection and antenatal diagnosis, upto date diagnostic tools of this hereditary coagulation disorder as well as management of haemophiliac patients in special situation like circumcision, road traffic accident, minor and major surgery.

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Introduction

The existence of life long bleeding disorders and their familial occurrence was noted very early in the medical literature by Alsaharavius during tenth century.[1] The medical practitioner's helplessness in the face of exsanguinating haemophilic blood loss profoundly impressed the early writers.

A full understanding of the pathophysiology and genetics of these disorders was long delayed by the complexities of the clotting mechanism. Recent advances in the protein chemistry and recombinant DNA technology have produced a comprehensive account both of normal coagulation and of the molecular genetics of some types of haemophilia.[2] Restriction fragment length polymorphisms are now routinely used to determine carrier state for haemophilia.

Hereditary disorders of coagulation usually are the results of a deficiency or abnormality of a single plasma protein. Although hereditary coagulation disorders are rare. Of these hemophilia is common. Three variants can be recognized.

1. Haemophilia A (Factor VIII deficiency) and haemophilia B (factor IX deficiency) are the most common (1 in 10,000 to 15,000 person) and serious hereditary coagulation factors deficiencies.[3]

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The clinical findings in haemophilia A & haemophilia B are virtually identical.

2. Haemophilia C refers to bleeding disorders associated with reduced level of factor XI

3. Parahaemophilia due to hereditary deficiency of factor V or labile factor or proaccelarin deficiency.

Haemophilia A is from four to eight times more common than haemophilia B.[4] Haemophilia is a hereditary bleeding disorder of male which is transmitted by healthy women. Almost but not quite all patients are male. However haemophilia has been documented in human females under following circumstances.[5]

1. The most common form is that seen in a minority of heterozygous carriers in whom X-chromosome inactivation may occur at an unusually early stage of embryogenesis and results in unusually low level of factor VIII.[6]

2. A second cause of female haemophilia is a mating between an affected male and a carrier female.[7]

3. In several other instances of female haemophilia, the disorders appeared to have developed presumably as the result of a newly mutant gene.[8]

4. Unusual degrees of X-chromosome inactivation ("hyperlyonization") may produce severe factor VIII deficiency in some carriers of haemophilia A.

Historical perspective:

Haemophilia has been recognized a clinical entity since Biblical times. Talmudic writings permitted the avoidance of circumcision when there was repeated history of death from circumcisional bleeding in male siblings. The disease was described clearly by Otto JC of France in 1839.[9] In 1893, Wright called attention to the prolonged coagulation time. Other investigators in this field were Addis, Sahli, Howell and Cekada of United Kingdom and Germany respectively.[10]

Haemophilia A: A severe and frequently fatal haemorrhagic diathesis that affected the male children of certain families.

Incidence: Haemophilia A has been recognized in all areas of the world. The disorder seems to be rare among Chinese and uncommon in Africans.[11] It's incidence has not been defined accurately; the best available estimates range from 1 in 20,000 to as high as 1 in 10,000 persons.[12]

Genetics: Haemophilia A is the classic example of an X-linked recessive trait. In such a disorder the defective gene is located on the X-chromosome.[13] In males who lack a normal allele, the defect is manifested by clinical haemophilia (generation I, number I). The affected male will not transmit the disorder to his sons (generation II, number 4 & 5), because his Y chromosome is normal. All of his daughters, however will be carriers of the trait, because they inherit his X chromosomes (generation II, number 2 &3). Most of these women will be unaffected clinically because of the presence of normal allele from mother. The female carrier will transmit the disorder to one half of her sons (generation III, number 6 &7) and the carrier state to one half of her daughters (generation III, number 8 & 9).

Pathophysiology: It is now generally accepted as first hypothesized by Addis in 1910, that fundamental abnormality in haemophilia A is a deficiency or abnormality of plasma protein known as antihaemophilic factor or factor VIII.[14] Factor VIII normally circulates in the plasma bound to a much larger molecule, the von Willebrand factor (vWF). The functional attribute of factor VIII that is essential in coagulation is designated VIIIc. Factor VIIIc and its functional and immunological properties are aberrant or deficient in patient with haemophilia. The vWF (formerely called VIIIag) acts as a carrier for factor VIII and is abnormal in von Willebrand disease. The production of vWF is coded by an autosomal gene, and this protein apparently is qualitatively normal and is present in normal or increased amount in patient with haemophilia A.[15]

Variants: Four variants of haemophilia A have been recognized:

1. CRM (Cross-reacting material) positive -Autologous antibodies that interact with vWF (formerely called VIIIag) an also cross-react with most haemophilic plasma

2. CRM (Cross-reacting material) negative – Autologous antibodies that interact with vWF (formerely called VIIag) do not cross-react with most haemophilic plasma.

3. Haemophilia A with autosomal dominant transmission has been reported.[16]
4. Heckathorn's disease- inherited as an X-linked recessive trait and is manifested by fluctuating deficiencies of VIIIc and persistent abnormalities of prothrombin consumption.[17] The basic defect in this disorder is one of the abnormal interaction between factor VIII and activated platelet membrane has been suggested.[18]

Clinical manifestations: The bleeding tendency usually appears infancy, but in mild cases it may not become apparent until adolescence or even adult life. The frequency and severity of bleeding is generally related to the blood level factor VIII.[19]

Three categories of severity may be distinguished arbitrarily.

1. Severe deficiency (Factor VIII level of 0 to 2U/dl), which manifested clinically by repeated and severe haemarthroses that almost invariably eventuate in crippling; such severe cases often are referred to as 'classic' haemophilia.
2. Moderate deficiency (factor VIII level of 2 to 5U/dl), which is associated with less frequent and less severe haemarthroses and seldom results in serious orthopaedic disability.
3. Mild deficiency (factor VIII level of 5 to 25U/dl), in which haemarthroses and spontaneous bleeding manifestation may be absent together; although serious bleeding may follow surgical procedure or traumatic injury.

Clinical severity, relative incidence and laboratory finding on Haemophilia A & Haemophilia B.

Severity	Factor VIII or IX level (U/dl)	Clinical picture	Relative incidence (% of cases)	Coagulation tests	
				Coagulation time	Partial Thromboplastin time
Severe	0-2	Haemarthrosis & spontaneous bleeding severe and frequent ; crippling common	50%	Prolonged	Prolonged
Moderate	5-Feb	Spontaneous bleeding & haemarthroses in-frequent; crippling uncommon; serious bleeding from trivial injuries.	30%	Normal	Prolonged
Mild	25-May	Spontaneous bleeding and haemarthroses uncommon; unsuspected and serious bleeding from traumatic injuries & surgery; diagnosis may be missed	20%	Normal	Variable

Wound bleeding is the characteristic symptoms of all haemophiliacs. It is usually slow and persist for days to weeks. About 30% of affected male infant with haemophilia bleed with circumcision. Although bleeding may occur in any area of the body, the hall mark of haemophilia is haemarthrosis. Bleeding into joint may be spontaneous or induced by minor trauma. The earliest joint haemorrhages appear most commonly in knee and ankle, because of the lack of stability of these joints as the toddler assumes an upright posture. Other joints that may be involved are the elbows, hips, wrists, shoulder and small joints of hand and feet. Physical examination reveals muscle spasm and limited motion of affected joint. The joint may be warm and grossly distended and discoloured.

Large ecchymoses and bleeding into tissues cause the formation of subcutaneous and intramuscular haematomas which vary in blood content from a few mililitre to several litres.

A tendency to skin bruise excessively after slight injury is noted by most haemophiliacs, but spontaneous bleeding into the skin and subcutaneous tissue is common only in severe deficiency.

Gastrointestinal and genitourinary bleeding: Haemorrhage from the mouth, gums, lips and frenulum of tongue is common and often serious. Haematemesis, malaena or both are not uncommon. Haemorrhage may be accompanied by abdominal pain, distension, increased peristalsis, fever, and leukocytosis. Bleeding from gums is uncommon during primary dentition but is sometimes troublesome during the sheeding of these teeth. Bleeding from the sockets after tooth extraction is almost invariable and in mild haemophiliacs is often the first and sometimes the only manifestation of the disease. In severe haemophilia, spontaneous epistaxis and bleeding into muscular tissues are not uncommon.

Haematuria although more common than gastrointestinal bleeding. The bleeding may arise in the bladder or in one or both kidneys and may persist for days or weeks. Clots in renal parenchyma present with colic.

Psoas and retroperitoneal haematomas: spontaneous haemorrhage into internal fascial spaces and muscles of the abdomen is common in haemophilia A. Bleeding into or around the iliopsoas muscle produces pain of progressively increasing severity and tenderness; when it occurs on the right side, it may closely simulate acute appendicitis. Retroperitoneal haemorrhage and intraperitoneal haemorrage are also common. Computerized tomography of abdomen may be helpful in the diagnosis of these haematomas.

Central nervous system bleeding: Life threatening bleeding in the haemophilic patient is caused by bleeding into the vital structures (e.g. central nervous system, and upper airways) or by exsanguination (external, gastrointestinal or iliopsoas haemorrhage). Intracranial haemorrhage either extra or intracerebral is not uncommon, particularly in severe haemophilia. In children, it most commonly occur after head injury, but in adults, it is usually spontaneous. Bleeding into spinal cord or canal is rare.

Haemophilia B (Synm: Christmas disease, factor IX deficiency and plasma thromboplastin component deficiency).

Haemophilia B was first distinguished from haemophilia A by Aggeler et al. in 1952.[20]

Variants: When plasma from patient with haemophilia B is tested against autologus antibodies, three distinct groups can be defined:

I) CRM positive variant - the most common

II) CRM negative variant - In one CRM negative variant, known as haemophilia B Leyden, the clinical manifestations tend to diminish with advancing age i association with a rise in factor IX level from as low as 1U/dl in childhood to levels of 20U/dl or more in adult life.[21] The Leyden variant has been characterised as CRM negative at birth but become CRM positive or CRM^R with advancing age.

III) CRM^R variant - In which antibody neutralization is variable in extent and approximately is proportional to coagulant factor IX activity.

In another variant of haemophilia B, the prothombin time is prolonged when performed with bovine brain thromboplastin. This disorder has been termed hemophilia Bm and is characterized by the presence in the plasma of CRM that neutralizes both autologus and heterologus antibodies to factor IX.[22]

Genetics: Haemophilia B is inherited as an X-linked recessive trait, but the locus on the X chromosome gene controlling factor IX production is remote from that involved with factor VIII biosynthesis.[23] Patient with haemophilia B who do not have a clear cut family history ("spontaneous" cases) are relatively uncommon.

Haemophilia C (Factor XI deficiency/plasma thromboplastin antecedent deficiency) was first recognized by Rosenthal et al in 1953.[24] This disorder was first to be transmitted as autosomal dominant trait with high degree of penetrance with variable expression. Later study suggested that it is transmitted as an incompletely recessive autosomal trait manifested either as a major defect in homozygous individuals with factor XI level below 20U/dl or as a minor defect in heterozygous individuals with level ranging from 30 to 65U/dl.[25] The incidence of this disorder compared to that of other hereditary coagulation disorder, varies widely, ranging from 1 to 18% and even more of surveyed cases. Haemophilia C is frequently encountered in Ashkenazi Jews but has been found in many other ethnic group. [26]

Clinical features:

The clinical manifestation of factor XI deficiency are milder than those of haemophilia A or B. Spontaneous bleeding is rare, and haemorrhage usually occurs after trauma or a surgical procedure. The bleeding associated with factor XI deficiency is not correlated with amount of factor XI. Some patients with severe deficiency may have minimal or no symptoms at the time of major surgery.

Haemarthrosis is uncommon, but delayed bleeding has been a particularly treacherous feature in some patient.

Laboratory diagnosis:

1. Quantitative assay for factor XI by using celite eluate test shows factor XI levels in the plasma are in the range of 3 to 15U/dl.
2. Partial thromboplastin time (PTT) and the coagulation time of whole blood are prolonged
3. Prothrombin consumption is deficient.
4. The bleeding time is rarely prolonged.

Parahaemophilia (Hereditary deficiency of factor V/labile factor/proaccelarin deficiency) is an uncommon disease that was first described by Owren in 1944.[27] It has been reported from various parts of the world and is transmitted as an autosomal recessive trait that manifest clinically only in individuals who inherit the defective gene from both parents.

Clinical features: The clinical manifestations of the disorder usually are relatively mild. Mildly affected patients experience spontaneous epistaxis, easy bruisability, menorrhagia and excessive bleeding after dental extraction or surgical procedures. In severely affected patients haematomas, spontaneous gingival bleeding and bleeding into gastrointestinal tract or central nervous system may occur.

Laboratory diagnosis:

I) The bleeding time is prolonged in approximately one third of patients - an observation that remains unexplained.

II) Factor V deficiency is the only disorder in which a prolonged prothrombin time is associated with deficient function of plasma reagent in thromboplastin generation test.

III) Specific assays for factor V are based on the prothrombin time and are easy to perform. Good results may be obtained by using aged oxalated or EDTA plasma as a factor V deficient substrate.

Diagnosis of haemophilia

The diagnosis can usually be suspected from clinical and hereditary features but is established with certainty only with the aid of laboratory tests.

I) Clinical and hereditary features: The clinical features of most importance are the sex, age of onset and the type of bleeding. The diagnosis is therefore strongly suggested by the onset in a male child of an abnormal bleeding tendency and a history of bleeding in male relatives on the maternal side of family.

In severe haemophilia, the onset of ecchymoses, prolonged bleeding from lacerations and other wounds, and bleeding into deep tissues and joints usually commences before the age of two years; on the other hand, in mild haemophilia symptoms may not appear until adolescence or adult life when

abnormal bleeding follows tooth extraction or surgery. The hereditary features of importance is evidence of sex-linked recessive inheritance. A detailed family history must be taken and a family tree drawn for accurate reference.

II) Laboratory findings:

a) Ancilliary coagulation tests.

1) One-stage technique for Factor VIII assays shows reduced level of Factor VIII and Factor IX in blood.

2) The whole blood coagulation time may be prolonged for 24 hours or longer and may show spontaneous irregular variation.

3) One stage test of prothrombin consumption is abnormal.

4) Thromboplastin generation test (TGT) may be abnormal. In unusual situation, a thromboplastin generation test could be used to distinguish between Factor VIII and Factor IX deficiency. TGT is also important to exclude the presence of a coagulation inhibitor.

5) The plasma recalcification time is greatly prolonged

6) Thrombin time and stypven time are normal.

7) Increased plasma level of $\alpha 2$ - macroglobulin and diminished level of heptogloblin have been described in haemophiliacs after replacement therapy.

8) Factor VIIIag (Cag) Assays by one stage technique demonstrate the absence of factor VIIIag in most severely affected haemphiliacs and correlate well to factor VIIIc measurement in most cases. Results are more variable when mild haemophilias are tested. In most serious, about 10% of patients have detectable level of factor VIIIag and a low VIIIc to VIIIag ratio.

b) Screening test of haemostasis and coagulation

1) The activated partial thromboplastin time (APTT) usually is prolonged in patient with all grades of haemophilia except the mildest, or when the patient has recently received transfusion with fresh blood. Abnormal results are obtained if the factor VIIIc level is less than 20 to 25% of normal.

2) One stage prothrombin time is normal

3) The platelets count and morphology are usually normal or elevated and clot retraction is normal.

4) The bleeding time and tourniquet test is usually normal. In some patient with haemophilia A, bleeding time may be prolonged and reaction to tourniquet test may be positive. This may be attributable to the use of aspirin, the administration of factor VIII containing blood products or to associated platelets dysfunction.

Laboratory findings in the common hereditary coagulation disorders

Disorder	Bleeding time	PTT	Prothrombin time	Coagulation consumption	Prothrombin	Thrombin time	Stypven time	TGT	Ancillary tests
Haemophilia A	N	A	N	A	A	N	N	PD	vWF normal or increased; ratio VIIIc/vWF low
Haemophilia B	N	A	N	A	A	N	N	SD	---
Haemophilia C (Factor XI deficiency)	N	A	N	A	A	N	N	Mild PD and SD	---
Parahaemophilia (Factor V deficiency)	uN	A	A	vA	vA	N	A	Mild PD	---

Key: N, normal; A, abnormal; D, deficient; P, plasma; S, Serum; V, Variable; U, Usually; PTT, Partial Thromboplastin time; TGT, Thromboplastin generation test.

The platelet count and clot retraction are normal in all uncomplicated hereditary coagulation disorder:

1) In severe deficiencies of coagulation factor, a prolonged bleeding time and a positive reaction to tourniquet test occasionally are found.
2) Test of prothrombin consumption and coagulation time yield abnormal results only in severe deficiencies.
3) Coagulation abnormalities are due to deficiency of factor VIIIc.
4) Patient's plasma may inhibit normal coagulation.
5) Abnormality may be corrected by increasing calcium concentration and may be magnified by diluting the thrombin solution.
6) Findings are significantly different in some variants.
7) Results of one- stage techniques may be uninterpretable.
8) Collection variable in degree.
9) Abnormality varies depending on techniques.

N.B. Stypven time: The one-stage prothrombin time "Performed with Russell's viper venom thus distinguishes between the deficiency of factor VII and deficiency factor IX.

Carrier detection and antenatal diagnosis:

Determination of carrier status in females depends on detailed information from the family history and results of coagulation factor assays.[28] Carrier could be diagnosed with reasonable confidence if the level of factor VIIIc was 50% or less of that expected from the level the factor vWF.

Carrier detection can be carried out using molecular genetic testing either by direct detection of mutation within the factor VIII gene or by indirect detection of abnormal gene using DNA polymorphisms within or adjacent to factor VIII gene as markers of the abnormal gene.[29]

Antenatal diagnosis can be undertaken in a female who has a high probability of being a carrier. This is accomplished by molecular analysis of faetal tissue obtained by chorionic villus biopsy at 9 to 11 weeks gestation.

Alternatively, the faetus can be sexed at 16 week's gestation by amniocentesis and if male, a faetal blood sample obtained at about 19 to 20 weeks.

Management of haemophiliac patients in special situations- circumcision, road traffic accident and minor and major surgery are as follows:

A. Management of patient with haemophilia A during circumcision and minor surgery are as follows.

1. In haemophiliac patients, general surgery should be carried out in a centre where there is laboratory facilities for coagulation factor assay, monitoring the response to replacement therapy (e.g. Factor VIII concentrate, cryoprecipitate) and detection of specific inhibitor of coagulation factor.
2. Patient should be tested before surgery for their response to DDAVP (1-deamino-8-D-arginine Vasopressin) by documenting factor VIII levels 30 to 60 minutes after treatment. This ensures that the drug will be effective in appropriate responders.
3. Circumcision and minor surgery in patients with haemophilia A can be done by parenteral and intranasal administration of non-blood-derived alternative DDAVP DAPAVP causes transiently increase in Factor VIII level two to three fold.
4. Antifibrinolytic agents like EACA/ε amino - caproic acid (6gm orally every 6 hours) or tranexamic acid (25mg/kg orally every 6 to 8 hours) has been administered together with DDAVP in an attempt to minimize fibrinolysis.
5. In minor bleeding (e.g. minor surgery and circumcision), a single dose of 10 to 15 U/kg of factor VIII concentrate is sufficient without any loading dose.

B. Management of haemophilia A patient with road traffic accident and major surgery are as follows:

1. Acute blood lose must always be replaced with adequate amount of blood either as whole blood or appropriate coagulation factor concentrate.

2. In the therapy for road traffic accident and major surgery in patient with haemophilia A, an initial loading dose of 3.5 bags/10kg of factor VIII concentrate should always be administered and sufficient factor VIII must be given often enough to ensure that the blood level does not fall below 30 to 50 U/dl for any length of time. Maintenance doses of 1.75 bags/10 kg usually are given every 8 to 12 hours.
3. The duration of replacement therapy depends on the cause of bleeding disorder, the severity of tissue damage and the response to treatment. Treatment should be continue for 10 to 14 days after road traffic accident and major surgery or until healing occurs.

C. Management of haemophilia B patients with major and minor haemorrhage are as follows:

1. In patients with haemophilia B, purified factor IX concentrates (e.g. Loading dose: 20-40 U/kg and maintenance dose: 15 U/kg every 24 hr. for 2 - 4 days) are recommended for the treatment road traffic accident and major and minor surgical procedures.
2. Recombinant factor IX is now available for clinical use.

D. Treatment of specific inhibitor of coagulation factor can be accomplished with immune tolerance regimens comprising of combination regimen of cyclo- phosphamide, intravenous immunoglobulin, high - dose factor VIII and immunoadsorption.

E. Local treatment at the bleeding site in haemophiliac patients are as follows:

1. Local pressure assists in arrest of bleeding and may be applied digitally, with pressure dressings and with sutures
2. Application of topical haemostatics (e.g. adrenaline solution in high concentration 0.5 - 1%) which may be used either alone or in combination with clotting agent, thrombin.
3. There should immobilization of the wound by bandaging and if necessary with splinting, helps to prevent recurrence of bleeding.

F. Gene therapy for inherited bleeding disorder is under active investigation. However haemophilia is an excellent model for gene therapy. Viral and non-viral constructs containing genes for factor VIII or factor IX have been used, as well as modified retrovirus, adenovirus or adeno-associated virus vectors.

At present time, there are three gene transfers trials in patients with haemophilia A & B and two are ongoing.

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